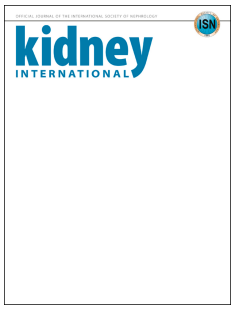

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Translational Science

How mTORC1 makes sense of nutrients

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Mammalian cells must sense and respond to fluctuations in environmental nutrient concentrations to preserve homeostasis.[1] A key step in this process is the recruitment of an ancient protein kinase called the mammalian Target of Rapamycin (mTOR) and its associated regulatory complex 1 (mTORC1) to the surface of the endolysosome — an organelle that functions in the degradation and recycling of cellular macromolecules.[1] There, the kinase activity of mTORC1 is initiated by its activator, the small GTPase Rheb, which conveys the second set of different stimuli[2] (e.g., cellular energy status, oxygen levels and growth factors; Figure 1a). Signalling from the endolysosome, mTORC1 sits at the centre of a regulatory network that balances anabolism and catabolism, guiding nearly every aspect of metabolic functions and ultimately coordinating cell and organism–wide growth.[3] In the kidney, appropriate regulation of mTOR is crucial for the homeostasis of kidney cells from the glomerular tuft along the entire nephron, ultimately regulating many fundamental processes from glomerular filtration barrier selectivity to tubular transport.[4] Dysregulated mTOR signalling disrupts kidney cell homeostasis and physiology, culminating in pathological processes such as the development of glomerular disease, polycystic kidney disease (PKD), acute kidney injury (AKI), and kidney transplant rejection.[4] Moreover, imbalances in mTOR activity in the kidney and other tissues can lead to metabolic dysfunction and maladaptation, overfeeding the overgrowth of cancers and the pathologies associated with ageing and metabolic disease.[1,4]

What did we know about sensing of nutrients?

Considerable evidence indicates that nutrients, including amino acids, glucose, and cholesterol, activate mTORC1 through a mechanism[1,5,6] that requires the Rag guanosine triphosphatases (GTPases). Rags assemble as obligate heterodimers composed of RagA or RagB (which are similar to each other and functionally redundant) complexed with RagC or RagD (also functionally equivalent), and the latter are tethered to the endolysosomal

membrane by the pentameric Ragulator complex. When the cells are nourished, the abundance of nutrients promotes the transition from the "inactive" combination of guanosine diphosphate (GDP)–loaded Rag A and guanosine triphosphate (GTP)–loaded Rag C to the "active" state of GTP–loaded RagA and GDP–loaded RagC.[1,5,6] This active configuration enables the Rag GTPase heterodimer to directly bind to the Raptor subunit of mTORC1 and anchor it to the membrane of the endolysosome for subsequent activation. Lacking a high–resolution view of the proteins' structure, the functional mechanisms regulating the capture of mTORC1 by the Rag GTPase heterodimer and its translocation to the endolysosome have remained poorly understood.

In a recent study[7] published in *Science,* Anandapadamanaban and colleagues close this gap in knowledge by employing a series of powerful structural biology methods to define the architecture of the human Rag GTPase heterodimers complexed with mTORC1. Using cryo–electron microscopy (EM) and molecular characterization, their work reveals the conformations of the Rag GTPase heterodimers that underlie nutrient sensing by mTORC1, suggesting that a particularly elaborate and stringent regulatory mechanism is at work on the surface of the endolysosome.

What did the study show?

mTORC1 and its partner Rag proteins are very dynamic, rapidly coming together and then falling apart, which greatly decreases the odds of capturing an intact complex. To turn the tables in their favour, the authors began their investigation by engineering a variety of single–amino acid genetic mutations into the Rag GTPases. These mutations alter the affinity for the guanine nucleotides and increase mTORC1 association with Rag GTPases, thereby enabling the components to linger together in a bound state for slightly longer than the wild–type proteins. This feat of molecular engineering allowed the researchers to view the three–dimensional shapes of proteins in their native cellular environment by EM and to

resolve the structure of the entire Rag–mTORC1 multiprotein complex at an extraordinary level of detail — roughly 5.5 angstroms.

With a detailed protein structure in hand, the authors were able to discern some key structural elements of the Rag–mTORC1 complex, as detailed in Figure 1b. Their EM reconstruction suggests that the central region of Raptor forms an extensive binding surface with the Rag GTPase heterodimer, interacting predominantly with the RagA GTPase. In contrast, the C–terminal regulatory domain, a large globular domain unique to Rag GTPases, points away from the Raptor subunit of mTORC1. The reverse states of Rags do not bind Raptor, because the GDP–loaded RagA would rearrange and disrupt the binding sites for Raptor, whereas the GTP–loaded RagC lacks residues analogous to the Raptor–binding residues of RagA. The binding of the RagA/RagC heterodimer to mTORC1 does not introduce any conformational changes within mTORC1, in line with the concept that Rag GTPase heterodimer might serve as platform for properly coupling mTORC1 to the endolysosome, facilitating the lasting activation of mTOR kinase by the Rheb GTPase.

Anandapadamanaban and colleagues not only identified the nucleotide–dependent conformations of the Rag GTPase heterodimers that are necessary for the binding to mTORC1, but also provided a framework for understanding the structural mechanisms underlying the intersubunit crosstalk within the Rag GTPase heterodimer. This intersubunit communication is a unique mode of regulation for GTPases and informs how the Rag GTPases transmit amino acid availability to mTORC1.[1] Specifically, when one subunit binds GTP, it triggers a conformational change that makes it dominant over the other and drives the Rag heterodimer into a locked conformation, preventing the association of a second GTP with non–dominant Rag. This locking mechanism is necessary for the normal response of mTORC1 to nutrient availability, providing a possible explanation for the evolutionary advantage of the heterodimeric architecture of the Rag GTPases. Consistent with this latter

idea, the structural studies revealed that local conformational changes take place in the switch machinery of the GTPase domain upon the binding of GTP to a subunit, ultimately making it less favourable for the heterodimer to accommodate the binding of GTP in both GTPase domains at the same time. These findings indicate that a conformational communication between RagA and RagC subunits within the heterodimer appears necessary for the binding of mTORC1 to the membrane of the endolysosome.

Why is the study important?

Combining this new evidence with previously published structures[8,9] for other mTORC1 pathway components, the authors propose a model in which the Rag GTPase heterodimers, which are localized to the endolysosomal membrane via binding to Ragulator, can be bound to mTORC1 at the same time as Rheb, which is tethered to the endomembrane through lipid–modified terminal residues. This interaction places mTORC1 on the endolysosome and facilitates the lasting activation of mTOR kinase. The intimate communication between the mTOR pathway and the endolysosome might serve as a "metabolic rheostat" that tunes the storage and mobilization of nutrients in response to ever–changing environmental conditions. Given that the dysregulated mTOR nutrient sensing pathway is a signature of many diseases of overfeeding — such as obesity and type 2 diabetes — it is tempting to imagine that excessive nutrients might force mTORC1 to remain in a persistent "on–state" by modulating the dynamics of the Rag GTPase heterodimer. This abnormal mTOR–driven nutrient response can further exacerbate metabolic dysregulation and disease. The structural insights presented by Anandapadamanaban and colleagues might therefore lead to new ways of designing small molecules that are more selective for mTORC1 signalling than existing drugs such as rapamycin, whose lack of specificity can be problematic from a therapeutic perspective — for example, causing unwanted, and often severe, side effects and toxicities.

By linking nutrient homeostasis, metabolism, and signal transduction, these studies are relevant for health and disease and could have implications beyond the hunt for the nutrient sensor.

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Figure 1. Regulation of mTORC1 signaling pathways at the surface of the endolysosome. (**a**) In response to nutrients, the Rag GTPases promote the translocation of mTORC1 to the membrane of the endolysosome, where the growth factor–induced transition from the inactive (GDP–loaded) form to the active GTP–bound Rheb promotes the lasting mTOR kinase activation. Amino acid abundance within the endolysosomal lumen, particularly arginine, is detected by the SLC38A9 amino acid transporter. The activation of mTORC1 by cholesterol also requires SLC38A9 in a manner that is separable from its arginine sensing function. Signalling from the endolysosome, mTOR activation initiates a downstream anabolic cascades that triggers the production of proteins, lipids, nucleotides, and other macromolecules while suppressing catabolic processes, such as autophagy and the endolysosome biogenesis. [Adapted with permission of Journal of Cell Biology from Lim C-Y and Zoncu R. The lysosome as a command-and-control center for cellular metabolism J Cell Biol (2016) 214 (6): 653–664; permission conveyed through Copyright Clearance Center] (10)

(**b**) Model of the mTORC1–Rheb–RagA/RagC–Ragulator complex on the endolysosomal surface. The mTOR complex 1 is nucleated by three components: mTOR, mammalian lethal with SEC13 protein 8 (mLST8) and its unique defining subunit, the scaffold regulatory–associated protein of mTOR (Raptor). Raptor–RagA/RagC was superimposed on Raptor in the mTORC1–Rheb complex.[8] The cysteine-rich domain of the mTORC1–RagA/RagC–Rheb was superimposed onto the cysteine-rich domain of the crystal

structure of the Ragulator–cysteine rich domain complex.[9] Under growth–promoting conditions (amino acid replete, plus growth factors), the active RagA/RagC heterodimers, which localize to endolysosome though the binding to Ragulator, and Rheb, which is associated with endomembrane through C–terminal farnesylation, can interact at the same with mTORC1, enabling its subsequent activation and signalling. Three α–helices within the α–solenoid domain of Raptor directly engage and form a network of hydrogen bonds and salt bridges with the switch machinery of the GTP–loaded RagA. Mutations that disrupted Rag–Raptor binding inhibit mTORC1 endolysosomal recruitment and signalling.
[From Anandapadamanaban, M. et al. Architecture of human Rag GTPase heterodimers and their complex with mTORC1. Science 2019; 366, 203-210 (7) Adapted with permission from AAAS.]